

AMENDMENTS TO THE SPECIFICATION

On page 10, please make the following amendments:

In carrying out the present assay in a microtiter plate format, a plasma sample is obtained from a patient and applied to a surface, such as a surface of a microtiter plate, that has been precoated with a secondary ~~anti-6-keto-~~ PGF_{1 α} antibody. An amount of anti-6-keto- PGF_{1 α} primary antibody and anti-6-keto- PGF_{1 α} -aequorin conjugate is then added to the plasma sample and the mixture incubated in the dark for a period of time ranging from about 1 to about 3 hours, preferably about 2 hours at room temperature. The surface is then washed to remove unbound primary antibody and conjugate, and the light intensity is determined and correlated with the amount of 6-keto- PGF_{1 α} present in the sample, which in turn directly correlates with the amount of prostacyclin in the sample.

On page 12, please make the following amendments:

The present invention also contemplates kits for measuring amount of prostacyclin in a plasma sample. Kits preferably contain a primary and secondary antibody for 6-keto- PGF_{1 α} and a secondary antibody and a 6-keto- PGF_{1 α} -aequorin conjugate. Preferably, the conjugate is a cysteine-free mutant of aequorin conjugated to 6-keto- PGF_{1 α} . The kit may also include assay buffer, deionized water, and/or microtiter plates or other surfaces pre-coated with the secondary antibody or for coating with secondary antibody at the time of performing the assay.

On Page 14, please replace the first full paragraph with the following:

Conjugation of Aequorin to 6-keto-Prostaglandin F_{1 α} : A volume of 1 mL of 1 x 10⁻⁶ M. aequorin was allowed to incubate with coelenterazine for 3 h at 4°C. The N-hydroxysuccinimide

ester of 6-keto-Prostaglandin $F_{1\alpha}$ was prepared by reacting with the water-soluble analog of *N*-hydroxysuccinimide (NHS), sulfo-NHS, and the dehydrating agent EDC.¹⁷ Grabarek, Z. a. G., J. Anal. Biochem. 1990, 185. The analyte 6-keto-Prostaglandin $F_{1\alpha}$ (1 mg/mL) was allowed to react with ~ 5 mM sulfo NHS and ~ 2 mM EDC (final concentrations) for 30 minutes at R.T. The reaction was quenched by adding β -mercaptoethanol to a final concentration of 20 mM. This NHS ester of the analyte was then conjugated to aequorin with bound coelenterazine in three different mole ratios, 1:50, 1:200, and 1:500 of aequorin: 6-keto- PGF $F_{1\alpha}$. The conjugates were passed through a size exclusion column in order to remove any unconjugated NHS ester of 6-keto- PGF $F_{1\alpha}$ and any residual reactants or reducing agent. The fractions were tested for activity of aequorin by taking 100 μ L of each fraction, then adding 3 μ L of 2.36×10^{-4} M coelenterazine and measuring the intensity of light emitted as described before. The fractions showing activity were combined and lyophilized. The lyophilized conjugates were resuspended in 30 mM Tris-HCl buffer, pH 7.0, containing 2 mM EDTA, 0.15 M NaCl and 1 mg/mL BSA (assay buffer) and stored in aliquots at -80°C.